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Large Granular Lymphocyte Leukemia With Pure Red Cell Aplasia in a Renal Transplant Recipient

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Neoplastic disorders sometimes accompany a renal transplant. Herein, we report a large granular lymphocyte (LGL) leukemia patient with pure red cell aplasia (PRCA) after renal transplantation. A 36-year-old female was presented to our department with anemia in February 1996. She had undergone hemodialysis because of pregnancy in December 1981. She received a renal transplantation from her mother in April 1986. After the transplantation, she received cyclosporin A (CyA) at 2 mg/kg/day, mizoribine at 1 mg/kg/day, and methylprednisolone at 0.1 mg/kg/day for 8 years. In July 1995, her hemoglobin level dropped to 9.3 g/dl, and anemia developed gradually. In January 1996, her hemoglobin was 5.8 g/dl, and she was given a red blood cell transfusion. Laboratory findings were as follows: RBC 1.46 × 10^{12}/L; hemoglobin 5.8 g/dl; hematocrit 17.8%; leucocytes 5.2 × 10^{9}/L with 62.4% neutrophils, 34.1% lymphocytes, 2.6% monocytes; platelets 50.8 × 10^{10}/L; reticulocytes 0.4%. Bone marrow aspirate smears and biopsy sections revealed normal myeloid and megakaryocyte differentiation with few erythroid precursors. The lymphocytes were of medium size with granules in the cytoplasm. More than 90% of lymphocytes were of the LGL type. Surface markers of peripheral blood mononuclear cells demonstrated increases in the $CD2^+$, $CD3^+$, $CD4^-$, and $CD8^+$ populations. A monoclonal rearrangement of T-cell receptor (TCR)-β chain gene was found by Southern blot analysis of the mononuclear cells in peripheral blood. A diagnosis of LGL leukemia with PRCA was made. During the next 4 months, she received six red blood cell transfusions, a total of 12 U. In March 1996, the patient was treated with cyclophosphamide (1 mg/kg/day). After 1 month of treatment, serum GPT levels increased to 60 IU/l. The dose of cyclophosphamide was reduced to 0.5 mg/kg/day. Two months after initiation of the therapy, the patient developed reticulocytosis and blood transfusion was not needed thereafter. During remission, the number of $CD2^+$, $CD3^+$, $CD4^-$, and $CD8^+$ lymphocytes decreased. Large granular lymphocytes decreased to less than 10% of peripheral blood. The monoclonal rearrangement of the TCR-β chain gene in peripheral blood disappeared. Am. J. Hematol. 57:72–76, 1998.

Key words: large granular lymphocyte leukemia; pure red cell aplasia; T cell receptor-β; cyclosporin-A

INTRODUCTION

The increased frequency of de novo malignancies occurring in transplant patients is documented [1,2]. Lymphoproliferative disease following renal transplantation might have been associated with immunosuppressive treatment with cyclosporin A (CyA) and EB virus infection [3–5].

Large granular lymphocyte (LGL) leukemia is a clonal disorder of T-cells or natural killer cells and is often associated with pure red cell aplasia (PRCA) [6,7]. CyA is an effective treatment for LGL leukemia and PRCA [7–14].

We report an interesting case of continuous CyA therapy and development of PRCA with LGL leukemia.

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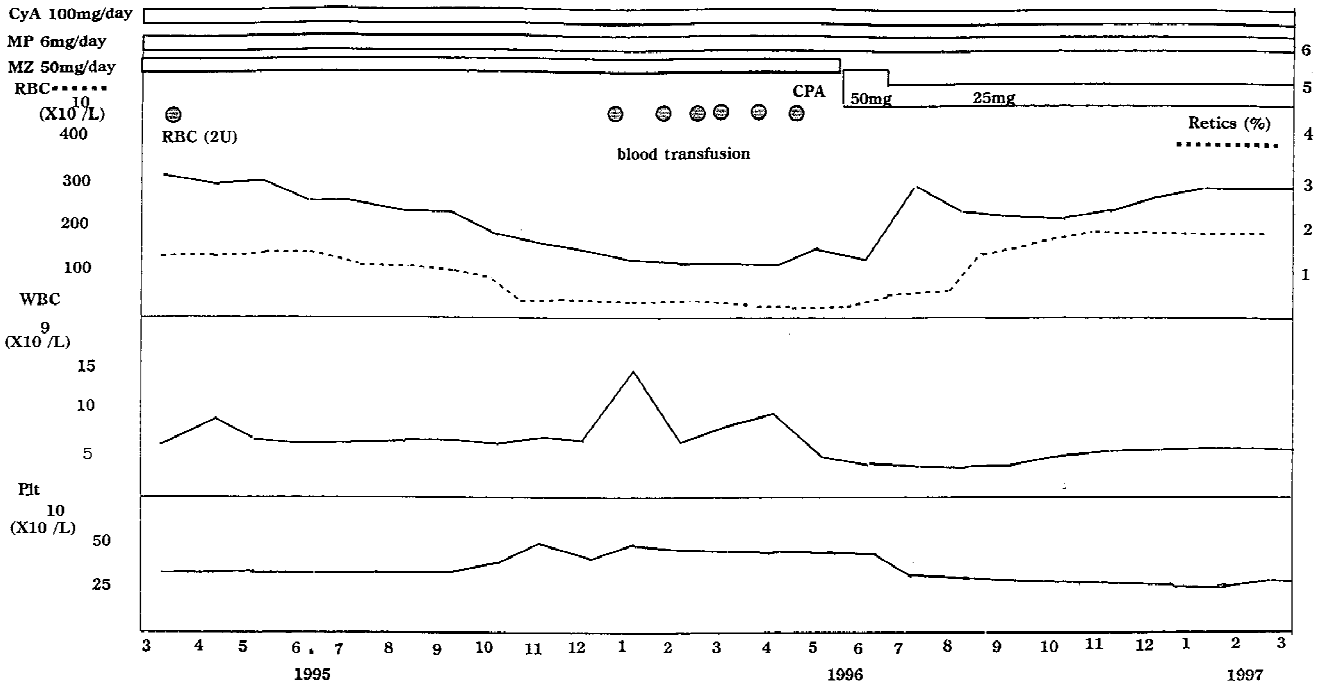

Fig. 1. Clinical course of a renal transplant patient with LGL leukemia and PRCA. MP, methylprednisolone; MZ, mizoribine; CPA, cyclophasphamide.

CASE REPORT

A 36-year-old female was presented to our department with anemia in February 1996. She had undergone hemodialysis because of pregnancy in December 1981, and had received a renal transplantation from her mother in April 1986. After the transplantation, she received cyclosporin A (CyA) at 2 mg/kg/day, mizoribine at 1 mg/kg/day, and methylprednisolone at 0.1 mg/kg/day for 8 years. By July 1995, her hemoglobin level had decreased to 9.3 g/dl, and anemia had developed (Fig. 1). In January 1996, her hemoglobin level was 5.8 g/dl, and she was given a red blood cell transfusion (2 U, one derived from 200 mL whole blood). Laboratory findings were as follows: RBC 1.46×10^{12}/L; hemoglobin 5.8 g/dl; hematocrit 17.8%; leucocytes 5.2×10^{9}/L with 62.4% neutrophils, 34.1% lymphocytes, 2.6% monocytes; platelets 50.8×10^{10}/L; reticulocytes 0.4% (Table I). Bone marrow aspirate smears and biopsy sections revealed normal myeloid and megakaryocyte differentiation with few erythroid precursors. Chromosomal analysis showed no abnormalities. The lymphocytes were of medium size with granules in the cytoplasm. More than 90% of lymphocytes were of the LGL type. Surface markers of peripheral blood mononuclear cells demonstrated increases in $CD2^+$, $CD3^+$, $CD4^-$, and $CD8^+$ populations (Table II). A monoclonal rearrangement of T-cell receptor (TCR)-β chain gene was found by Southern blot analysis [15] of the mononuclear cells in peripheral blood (Fig. 2). A diagnosis of LGL leukemia with PRCA was made. During the next 4 months, she received six red blood cell transfusions, a total of 12 U. In March 1996, the patient was treated with cyclophosphamide (1 mg/kg/day). After 1 month of treatment, GPT levels increased to 60 IU/l. The dose of cyclophosphamide was reduced to 0.5 mg/kg/day. Two months after initiation of the therapy, the patient developed reticulocytosis and blood transfusion was not needed thereafter. During remission, the number of $CD2^+$, $CD3^+$, $CD4^-$, and $CD8^+$ lymphocytes de-

TABLE I. Laboratory Findings of the Patient

Serum chemistry	
T-P	7.4 g/dl
T-Bil	0.6 mg/dl
GOT	17 KU
GPT	19 KU
ALP	170 KU
BUN	23.6 mg/dl
CREA	1.44 mg/dl
EPO	2,130 mU/ml
Serological test	
CRP	(−)
ANF	(−)
A-DNA	(−)
HTLV-1	(−)
HIV	(−)
Bone marrow examination	
NCC	8.8×10^4/μl
MgK	31.2/μl
Myeloid lineage	68.7%
Lymphoid lineage	26.1%
Erythroid lineage	2.4%

TABLE II. Surface Marker Analysis of Peripheral Blood Mononuclear Cells

	Initial presentation (1995.1.11) (%)	Remission with cyclophosphamide (1995.9.3) (%)
CD1	0.1	0.1
CD2	99.7	94.9
CD3	97.7	85.0
CD4	3.2	33.2
CD5	42.9	72.7
CD7	79.8	84.3
CD8	88.1	51.9
CD10	1.4	1.4
CD11b	6.1	3.1
CD13	2.0	0.9
CD14	2.4	2.6
CD16	6.1	2.0
CD19	2.4	0.2
CD20	0.2	4.5
CD25	0.3	0.7
CD33	1.0	0.5
CD56	3.2	2.5
CD57	14.1	14.9
HLA-DR	35.9	34.7

creased (Table II). Large granular lymphocytes made up less than 10% of peripheral blood. The monoclonal rearrangement of the TCR-β chain gene in peripheral blood disappeared (Fig. 2). This study was approved by the Investigational Review Board of the Tokyo Women's Medical College. Bone marrow aspirates and peripheral blood were obtained with informed consent.

DISCUSSION

Organ allograft recipients are at an increased risk of neoplasia. In 918 European renal transplant patients, the cumulative risk of neoplasia over 20 years rose to 40% compared with 6% in the age-matched control population [1]. Immunosuppressive therapy with CyA A and Epstein-Barr (EB) virus infection increases the risk of post-transplant lymphomas [2–5]. In this regard, the occurrence of LGL leukemia in this case may relate to the use of CyA because EB virus infection was not confirmed by the analysis with anti-EB virus antibody.

The diagnostic criteria of LGL leukemia is a granular lymphocytosis greater than 2,000/μl lasting for more than 6 months [6,7,16]. In the present case, 6 months before diagnosis of LGL leukemia, the absolute granular lymphocyte count was less than 2,000/μl. During this period, the patient's hemoglobin level decreased from 10.7 to 5.7 g/dl and she needed red blood cell transfusions. Semenzato et al. reported that patients with a relatively low absolute number of granular lymphocytes resemble those with greater than 2,000 granular lymphocytes/μl [17]. In the present case, LGL leukemia was diagnosed based on granular lymphocyte morphology, surface marker analysis, and T cell receptor gene assay.

Acquired secondary PRCA has been associated with thymoma, hematological malignancies, non-hematological solid tumor, infections, drug and chemical exposure, hemolytic anemia, collagen disease, pregnancy, severe renal failure, and severe nutritional deficiency. Most patients with chronic PRCA have autoimmune disease either due to autoantibodies to erythroid progenitor cells or to T-cell autoimmunity [18]. Chronic parvovirus B-19 infection in the immunodeficient state is a common cause of PRCA [19,20]. Our patient underwent immunosuppressive therapy with CyA after renal transplantation. An IgM serological test for Parvovirus was negative. While CyA treatment continued, her anemia recovered. Taken together, Parvovirus B-19 infection could not be the cause of PRCA in the present case.

Immune suppressive agents, such as CyA, cyclophosphamide, methotrexate, prednisolone, antithymocyte globulin, myleran, azathiopurine, and chrombucil, are the treatment options for patients with PRCA and LGL leukemia. CyA is often used in these patients [7–14], and as an immunosuppressive agent, is believed to inhibit the production of interleukin (IL)-2 and γ interferon (IFN) by T-lymphocytes, as well as the activation of cytotoxic T-cells by IL-2 [21]. Both IL-2 and γ IFN have been shown to inhibit the growth of hematopoietic progenitor cells [22]. In the present case, as the CyA trough level was 61.4 ± 15.3 (range: 38–83) ng/ml 6 months before the initial presentation of PRCA, malabsorption of drug could be excluded as a cause of PRCA. There are some reports that CyA is effective in treating LGL leukemia. Oshimi et al. studied 33 patients with LGL leukemia and reported that one patient with PRCA responded to CyA treatment [7]. Kouides et al. reported that LGL leukemia and PRCA were improved by CyA [8]. Jakubowski et al. used a combination of CyA and G-CSF to treat a patient with LGL leukemia and neutropenia [11], and observed a recovery in the neutrophil count. We previously reported on a PRCA patient with thymoma in whom the T-cell receptor β gene of mononuclear cells from both thymus and peripheral blood were rearranged. Though initially refractory to corticosteroid and cyclophosphamide, PRCA was successfully treated with CyA [23]. Raghavachar looked at cases of PRCA treated with CyA reported in the literature and calculated the overall response rate to be 65% [12]. Means et al. used a combination of CyA and corticosteroids and obtained responses in six of nine heavily pretreated patients [13]. Lacy et al. concluded that CyA was more effective than corticosteroids, cyclophosphamide, androgens, and antithymocyte globulin in the treatment of PRCA [10]. Four out of five patients with PRCA who were previously treated with corticosteroids and cyclophosphamide recovered following CyA treatment. Most LGL leukemia

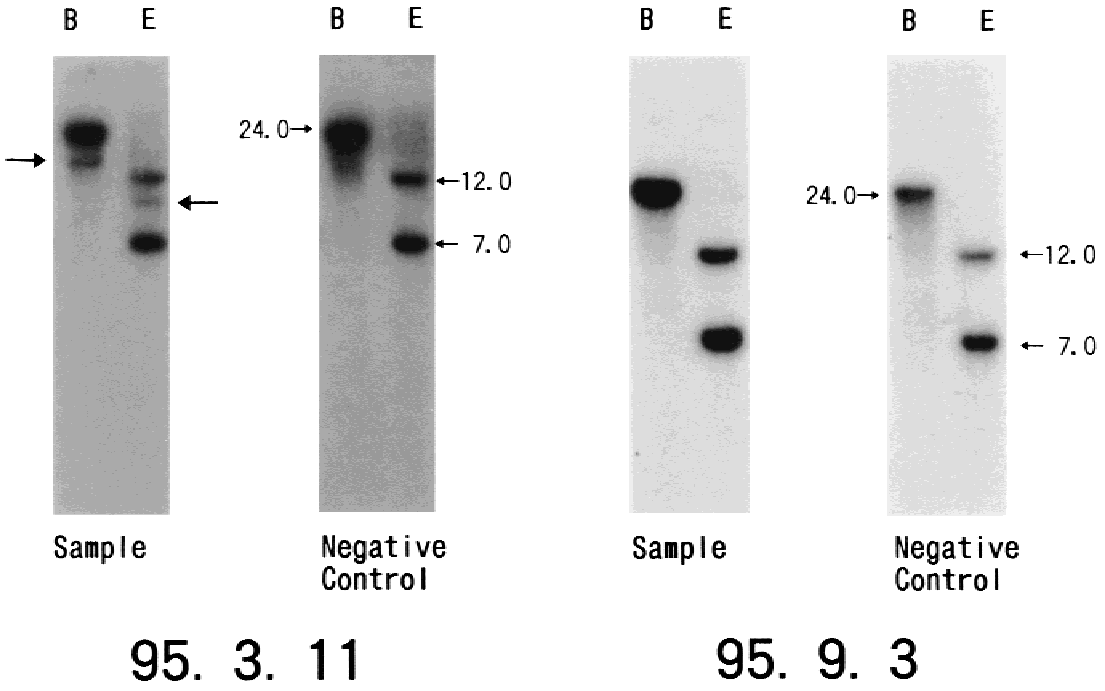

Fig. 2. Gene analysis. DNA samples (5 μg) were digested with Bam HI and EcoRV. Southern blot analysis of DNA was performed with TCR $C\beta_1$ probe. Lines on the right indicate the germline position. 24.0 kb is the germline position for Bam HI; 12.0 and 7.0 kb are the germline positions for EcoRV. The position of the rearranged fragment is marked by an arrow. The control (C) human placental DNA exhibits germline configuration. B: Bam HI; E: EcoRV; PB: peripheral blood; C: control DNA.

cases are clonal disorders with rearrangement of TCR and many are associated with PRCA [6,7]. Though as a renal transplant recipient, our patient had been treated with CyA, she developed LGL leukemia with PRCA. Interestingly CyA, one of the most effective drugs for PRCA available, did not inhibit the occurrence of PRCA accompanying LGL leukemia. PRCA with LGL leukemia might exhibit a heterogeneous response to CyA. In organ transplant patients treated with CyA, complications of LGL leukemia or PRCA might be a cause of anemia.

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